WEST Search History

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DATE: Thursday, February 22, 2007

Hide?	Set Name	Query	Hit Count
	DB=PGPB,USPT,EPAB; PLUR=YES; OP=ADJ		
☐	L45	L43 and enzym$.clm.	10
☐	L44	L43 and enzyme.clm.	10
☐	L43	L42 and l41	18
☐	L42	l5.clm.	34875
☐	L41	L40 and antibod$.clm.	39
☐	L40	l1 and l2	116
☐	L39	l32 and sweatner.clm.	0
☐	L38	l32 and sweetner.clm.	0
☐	L37	l32 and seetner.clm.	0
☐	L36	L35 and galactose	62
☐	L35	L34 and antibod$	62
☐	L34	L32 not @py>1993	276
☐	L33	L32 not @ay>1993	346
☐	L32	l30 and L31	1515
☐	L31	composition.clm.	416983
☐	L30	galactose.clm.	3410
☐	L29	L28 and galactose	1
☐	L28	5632990.pn.	1
☐	L27	L23 not @ay>1995	6
☐	L26	L23 not @ay>1993	2
☐	L25	L23 and L5	128
☐	L24	L23 not @py>1993	2
☐	L23	L22 and L21	131
☐	L22	mannose or galactose or acetylglycosamine or acetyllactose or glucose or fucose	178013
☐	L21	L20 and CEA	158
☐	L20	L19 and L16	1187
☐	L19	$glucuronidase	10866
☐	L18	L17 and L7	3
☐	L17	L16 not @ay>1993	3514

☐	L16	antibod$ NEAR2 enzyme	24424
☐	L15	5632990.pn.	1
☐	L14	L13 and CEA	0
☐	L13	L12 not @py>1993	7
☐	L12	L11 and L5	437
☐	L11	L10 and L7	513
☐	L10	enzyme and antibod$	138108
☐	L9	L8 not @ay>1993	3
☐	L8	L7 and L6	151
☐	L7	glucoronidase	663
☐	L6	L5 and L4	32358
☐	L5	conjugate or fusion	239183
☐	L4	L3 and antibod$	41141
☐	L3	enzyme and L2	46628
☐	L2	glycoprotein or glyclopolypeptide	56461
☐	L1	(bosslet or Czech or hoffman).in.	7326

END OF SEARCH HISTORY

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FILE 'HOME' ENTERED AT 11:01:16 ON 22 FEB 2007

=> file medline
COST IN U.S. DOLLARS

	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	0.21	0.21

FILE 'MEDLINE' ENTERED AT 11:01:24 ON 22 FEB 2007

FILE LAST UPDATED: 21 Feb 2007 (20070221/UP). FILE COVERS 1950 TO DATE.

All regular MEDLINE updates from November 15 to December 16 have been added to MEDLINE, along with 2007 Medical Subject Headings (MeSH(R)) and 2007 tree numbers.

The annual reload will be available in early 2007.

This file contains CAS Registry Numbers for easy and accurate substance identification.

```
=> s antibod?
L1      733926 ANTIBOD?

=> s enzyme?
L2      805235 ENZYME?

=> s l2 and l1
L3      106352 L2 AND L1

=> s conjugat? or fusion or link? or coupl?
         84773 CONJUGAT?
        144879 FUSION
          9402 FUSIONS
        148875 FUSION
                 (FUSION OR FUSIONS)
        442643 LINK?
        180421 COUPL?
L4      808454 CONJUGAT? OR FUSION OR LINK? OR COUPL?

=> s l4 (L) l3
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L4 (L) L3'
L5       66727 L4 (L) L3

=> s l4 and l3
L6       66727 L4 AND L3

=> s ?glururonidase
L7           1 ?GLURURONIDASE

=> s glucuronidase
         14401 GLUCURONIDASE
           295 GLUCURONIDASES
L8       14452 GLUCURONIDASE
                 (GLUCURONIDASE OR GLUCURONIDASES)
```

PubMed ID: 1739623

TITLE:	Molecular and functional characterisation of a fusion protein suited for tumour specific prodrug activation.
AUTHOR:	Bosslet K; Czech J; Lorenz P; Sedlacek H H; Schuermann M; Seemann G
CORPORATE SOURCE:	Research Laboratory of Behringwerke, Marburg, Germany.
SOURCE:	British journal of cancer, (1992 Feb) Vol. 65, No. 2, pp. 234-8. Journal code: 0370635. ISSN: 0007-0920.
PUB. COUNTRY:	ENGLAND: United Kingdom
DOCUMENT TYPE:	Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:	English
FILE SEGMENT:	Priority Journals
ENTRY MONTH:	199203
ENTRY DATE:	Entered STN: 10 Apr 1992 Last Updated on STN: 10 Apr 1992 Entered Medline: 24 Mar 1992

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NEWS	1		Web Page URLs for STN Seminar Schedule - N. America
NEWS	2		"Ask CAS" for self-help around the clock
NEWS	3	OCT 23	The Derwent World Patents Index suite of databases on STN has been enhanced and reloaded
NEWS	4	OCT 30	CHEMLIST enhanced with new search and display field
NEWS	5	NOV 03	JAPIO enhanced with IPC 8 features and functionality
NEWS	6	NOV 10	CA/CAplus F-Term thesaurus enhanced
NEWS	7	NOV 10	STN Express with Discover! free maintenance release Version 8.01c now available
NEWS	8	NOV 20	CA/CAplus to MARPAT accession number crossover limit increased to 50,000
NEWS	9	DEC 01	CAS REGISTRY updated with new ambiguity codes
NEWS	10	DEC 11	CAS REGISTRY chemical nomenclature enhanced
NEWS	11	DEC 14	WPIDS/WPINDEX/WPIX manual codes updated
NEWS	12	DEC 14	GBFULL and FRFULL enhanced with IPC 8 features and functionality
NEWS	13	DEC 18	CA/CAplus pre-1967 chemical substance index entries enhanced with preparation role
NEWS	14	DEC 18	CA/CAplus patent kind codes updated
NEWS	15	DEC 18	MARPAT to CA/CAplus accession number crossover limit increased to 50,000
NEWS	16	DEC 18	MEDLINE updated in preparation for 2007 reload
NEWS	17	DEC 27	CA/CAplus enhanced with more pre-1907 records
NEWS	18	JAN 08	CHEMLIST enhanced with New Zealand Inventory of Chemicals
NEWS	19	JAN 16	CA/CAplus Company Name Thesaurus enhanced and reloaded
NEWS	20	JAN 16	IPC version 2007.01 thesaurus available on STN
NEWS	21	JAN 16	WPIDS/WPINDEX/WPIX enhanced with IPC 8 reclassification data
NEWS	22	JAN 22	CA/CAplus updated with revised CAS roles
NEWS	23	JAN 22	CA/CAplus enhanced with patent applications from India
NEWS	24	JAN 29	PHAR reloaded with new search and display fields
NEWS	25	JAN 29	CAS Registry Number crossover limit increased to 300,000 in multiple databases
NEWS	26	FEB 13	CASREACT coverage to be extended
NEWS	27	Feb 15	PATDPASPC enhanced with Drug Approval numbers
NEWS	28	Feb 15	RUSSIAPAT enhanced with pre-1994 records

NEWS EXPRESS NOVEMBER 10 CURRENT WINDOWS VERSION IS V8.01c, CURRENT MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP), AND CURRENT DISCOVER FILE IS DATED 25 SEPTEMBER 2006.

NEWS HOURS	STN Operating Hours Plus Help Desk Availability
NEWS LOGIN	Welcome Banner and News Items
NEWS IPC8	For general information regarding STN implementation of IPC 8
NEWS X25	X.25 communication option no longer available

Enter NEWS followed by the item number or name to see news on that specific topic.

```
=> s ?glucuronidase
L9       14419 ?GLUCURONIDASE

=> s l9 and l6
L10         97 L9 AND L6

=> s CEA or (Carcinogenic Embryonic Antigen)
         11451 CEA
           376 CEAS
         11479 CEA
                 (CEA OR CEAS)
         19461 CARCINOGENIC
             6 CARCINOGENICS
         19466 CARCINOGENIC
                 (CARCINOGENIC OR CARCINOGENICS)
        100070 EMBRYONIC
             6 EMBRYONICS
        100072 EMBRYONIC
                 (EMBRYONIC OR EMBRYONICS)
        372385 ANTIGEN
        450906 ANTIGENS
        633561 ANTIGEN
                 (ANTIGEN OR ANTIGENS)
             2 CARCINOGENIC EMBRYONIC ANTIGEN
                 (CARCINOGENIC(W)EMBRYONIC(W)ANTIGEN)
L11      11481 CEA OR (CARCINOGENIC EMBRYONIC ANTIGEN)

=> s l11 and l10
L12          2 L11 AND L10

=> d ibib 1-2

L12  ANSWER 1 OF 2     MEDLINE on STN
ACCESSION NUMBER:    1998404122     MEDLINE
DOCUMENT NUMBER:     PubMed ID: 9733483
TITLE:               Prodrugs of anthracyclines for use in antibody
                     -directed enzyme prodrug therapy.
AUTHOR:              Florent J C; Dong X; Gaudel G; Mitaku S; Monneret C; Gesson
                     J P; Jacquesy J C; Mondon M; Renoux B; Andrianomenjanahary
                     S; Michel S; Koch M; Tillequin F; Gerken M; Czech J; Straub
                     R; Bosslet K
CORPORATE SOURCE:    UMR 176 CNRS/Institut Curie, Section Recherche, 26 rue
                     d'Ulm, F-75248 Paris Cedex 05, France.
SOURCE:              Journal of medicinal chemistry, (1998 Sep 10) Vol. 41, No.
                     19, pp. 3572-81.
                     Journal code: 9716531. ISSN: 0022-2623.
PUB. COUNTRY:        United States
DOCUMENT TYPE:       Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:            English
FILE SEGMENT:        Priority Journals
ENTRY MONTH:         199810
ENTRY DATE:          Entered STN: 20 Oct 1998
                     Last Updated on STN: 20 Oct 1998
                     Entered Medline: 8 Oct 1998

L12  ANSWER 2 OF 2     MEDLINE on STN
ACCESSION NUMBER:    92153684     MEDLINE
DOCUMENT NUMBER:     PubMed ID: 1739623
TITLE:               Molecular and functional characterisation of a
                     fusion protein suited for tumour specific prodrug
                     activation.
AUTHOR:              Bosslet K; Czech J; Lorenz P; Sedlacek H H; Schuermann M;
                     Seemann G
CORPORATE SOURCE:    Research Laboratory of Behringwerke, Marburg, Germany.
```

SOURCE: British journal of cancer, (1992 Feb) Vol. 65, No. 2, pp. 234-8.
Journal code: 0370635. ISSN: 0007-0920.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199203
ENTRY DATE: Entered STN: 10 Apr 1992
Last Updated on STN: 10 Apr 1992
Entered Medline: 24 Mar 1992

=> d ibib abs 2

L12 ANSWER 2 OF 2 MEDLINE on STN
ACCESSION NUMBER: 92153684 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1739623
TITLE: Molecular and functional characterisation of a fusion protein suited for tumour specific prodrug activation.
AUTHOR: Bosslet K; Czech J; Lorenz P; Sedlacek H H; Schuermann M; Seemann G
CORPORATE SOURCE: Research Laboratory of Behringwerke, Marburg, Germany.
SOURCE: British journal of cancer, (1992 Feb) Vol. 65, No. 2, pp. 234-8.
Journal code: 0370635. ISSN: 0007-0920.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199203
ENTRY DATE: Entered STN: 10 Apr 1992
Last Updated on STN: 10 Apr 1992
Entered Medline: 24 Mar 1992

AB A fusion protein consisting of the humanised Fab fragment of the anti CEA MAb BW 431 and the human beta-glucuronidase was expressed in BHK cells. Functional testing revealed that the specificity and avidity of the humanised V region was similar to the original murine MAb BW 431. Furthermore, the enzymatic activity, pH sensitivity and stability of the human beta-glucuronidase in the fusion protein was comparable to the activity of recombinant human beta-glucuronidase. Using anti-idiotype affinity chromatography, two molecules of a molecular weight of 125 kDa or 250 kDa could be visualized under nonreducing conditions in SDS-PAGE. Reducing conditions revealed a 25 kDa light and 100 kDa heavy chain. Due to its suitable biological characteristics this fusion protein might be an appropriate molecule allowing a site specific antibody directed enzyme prodrug therapy (ADEPT) in vivo.

=> d his

(FILE 'HOME' ENTERED AT 11:01:16 ON 22 FEB 2007)

FILE 'MEDLINE' ENTERED AT 11:01:24 ON 22 FEB 2007
L1 733926 S ANTIBOD?
L2 805235 S ENZYME?
L3 106352 S L2 AND L1
L4 808454 S CONJUGAT? OR FUSION OR LINK? OR COUPL?
L5 66727 S L4 (L) L3
L6 66727 S L4 AND L3
L7 1 S ?GLURURONIDASE
L8 14452 S GLUCURONIDASE

```
L9        14419 S ?GLUCURONIDASE
L10          97 S L9 AND L6
L11       11481 S CEA OR (CARCINOGENIC EMBRYONIC ANTIGEN)
L12           2 S L11 AND L10

=> s l3 and l9
L13         304 L3 AND L9

=> s l13 and l11
L14           2 L13 AND L11

=> file pctfull
COST IN U.S. DOLLARS                                  SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                       3.68       3.89

FILE 'PCTFULL' ENTERED AT 11:05:55 ON 22 FEB 2007
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FILE LAST UPDATED:          20 FEB 2007      <20070220/UP>
MOST RECENT UPDATE WEEK:        200707        <200707/EW>
FILE COVERS 1978 TO DATE

>>> IMAGES ARE AVAILABLE ONLINE AND FOR EMAIL-PRINTS <<<

=> s antibod?
L15       95616 ANTIBOD?

=> s ?glucuronidase
L16        6166 ?GLUCURONIDASE

=> s enzyme?
L17      135831 ENZYME?

=> s l15 (4W) l17
L18       11277 L15 (4W) L17

=> s conjugat? or fusion or link? or coupl?
          82444 CONJUGAT?
          84421 FUSION
          16216 FUSIONS
          85622 FUSION
                  (FUSION OR FUSIONS)
         327777 LINK?
         363802 COUPL?
L19      576301 CONJUGAT? OR FUSION OR LINK? OR COUPL?

=> s l19 and l18
L20       10996 L19 AND L18

=> s l20 and l16
L21        1160 L20 AND L16

=> s CEA or (Carcinogenic Embryonic Antigen)
           4397 CEA
             83 CEAS
           4455 CEA
                  (CEA OR CEAS)
           4047 CARCINOGENIC
            115 CARCINOGENICS
           4157 CARCINOGENIC
                  (CARCINOGENIC OR CARCINOGENICS)
          23813 EMBRYONIC
              2 EMBRYONICS
```

```
          23814 EMBRYONIC
                (EMBRYONIC OR EMBRYONICS)
          59847 ANTIGEN
          40235 ANTIGENS
          65876 ANTIGEN
                (ANTIGEN OR ANTIGENS)
             18 CARCINOGENIC EMBRYONIC ANTIGEN
                (CARCINOGENIC(W)EMBRYONIC(W)ANTIGEN)
L22        4458 CEA OR (CARCINOGENIC EMBRYONIC ANTIGEN)

=> s l22 and l21
L23          80 L22 AND L21

=> s l23 and galactose
          16533 GALACTOSE
            191 GALACTOSES
          16548 GALACTOSE
                (GALACTOSE OR GALACTOSES)
L24          28 L23 AND GALACTOSE

=> s l24 not py>1993
        1073184 PY>1993
L25           2 L24 NOT PY>1993

=> d ibib 1-2

L25    ANSWER 1 OF 2     PCTFULL   COPYRIGHT 2007 Univentio on STN
ACCESSION NUMBER:        1993002703 PCTFULL  ED 20020513
TITLE (ENGLISH):         PRODRUGS ACTIVATED BY TARGETED CATALYTIC PROTEINS
TITLE (FRENCH):          PROMEDICAMENTS ACTIVES PAR DES PROTEINES CATALYTIQUES
                         CIBLEES
INVENTOR(S):             KENTEN, John, Henry;
                         VON BORSTEL, Reid;
                         CASADEI, Jan, M.;
                         KAMIREDDY, Balreddy;
                         MARTIN, Mark, T.;
                         MASSEY, Richard, J.;
                         NAPPER, Andrew, D.;
                         SIMPSON, David, M.;
                         SMITH, Rodger, G.;
                         TITMAS, Richard, C.;
                         WILLIAMS, Richard, O.
PATENT ASSIGNEE(S):      IGEN, INC.
LANGUAGE OF PUBL.:       English
DOCUMENT TYPE:           Patent
PATENT INFORMATION:
                         NUMBER              KIND     DATE
                         ---------------------------------
                         WO 9302703           A1 19930218
DESIGNATED STATES
       W:                AU CA JP KR AT BE CH DE DK ES FR GB GR IE IT LU MC NL
                         SE
PRIORITY INFO.:          US 1991-740,501         19910805
                         US 1991-773,042         19911010
                         US 1992-919,851         19920731
APPLICATION INFO.:       WO 1992-US6530       A  19920804

L25    ANSWER 2 OF 2     PCTFULL   COPYRIGHT 2007 Univentio on STN
ACCESSION NUMBER:        1979000882 PCTFULL  ED 20020506
TITLE (ENGLISH):         MACROMOLECULAR ENVIRONMENT CONTROL IN SPECIFIC RECEPTOR
                         ASSAYS
TITLE (FRENCH):          CONTROLE D'UN MILIEU MACROMOLECULAIRE DANS DES
                         RECEPTEURS D'ESSAI SPECIFIQUES
INVENTOR(S):             LITMAN D;
```

WIFE R;
ULLMAN E;
HAREL Z;
MAGGIO E
PATENT ASSIGNEE(S): SYVA CO
LANGUAGE OF PUBL.: English
DOCUMENT TYPE: Patent
PATENT INFORMATION:

NUMBER	KIND	DATE
WO 7900882	A1	19791101

DESIGNATED STATES
W: BR JP SE CH DE FR GB
PRIORITY INFO.: US 1978-893650 19780405
US 1978-964099 19781124
APPLICATION INFO.: WO 1979-US202 A 19790402

=> d ibib abs kwic

L25 ANSWER 1 OF 2 PCTFULL COPYRIGHT 2007 Univentio on STN
ACCESSION NUMBER: 1993002703 PCTFULL ED 20020513
TITLE (ENGLISH): PRODRUGS ACTIVATED BY TARGETED CATALYTIC PROTEINS
TITLE (FRENCH): PROMEDICAMENTS ACTIVES PAR DES PROTEINES CATALYTIQUES CIBLEES
INVENTOR(S): KENTEN, John, Henry;
VON BORSTEL, Reid;
CASADEI, Jan, M.;
KAMIREDDY, Balreddy;
MARTIN, Mark, T.;
MASSEY, Richard, J.;
NAPPER, Andrew, D.;
SIMPSON, David, M.;
SMITH, Rodger, G.;
TITMAS, Richard, C.;
WILLIAMS, Richard, O.
PATENT ASSIGNEE(S): IGEN, INC.
LANGUAGE OF PUBL.: English
DOCUMENT TYPE: Patent
PATENT INFORMATION:

NUMBER	KIND	DATE
WO 9302703	A1	19930218

DESIGNATED STATES
W: AU CA JP KR AT BE CH DE DK ES FR GB GR IE IT LU MC NL SE
PRIORITY INFO.: US 1991-740,501 19910805
US 1991-773,042 19911010
US 1992-919,851 19920731
APPLICATION INFO.: WO 1992-US6530 A 19920804

ABEN Disclosed and claimed are prodrugs activated by catalytic proteins, such as enzymes and catalytic antibodies. The invention comprehends such prodrugs, as well as haptens, to elicit catalytic antibodies to activate the prodrugs. The prodrugs are useful as cytotoxic chemotherapeutic agents, e.g., as antitumor agents.

ABFR L'invention concerne des promedicaments actives par des proteines catalytiques telles que des enzymes et des anticorps catalytiques. L'invention concerne lesdits promedicaments, ainsi que des haptenes destines a induire les anticorps catalytiques a activer les promedicaments. Lesdits promedicaments sont utiles comme agents chimiotherapeutiques

cytotoxiques par exemple comme agents
antitumoraux.

DETD . . . Senter, et al., Patent Application EP 88112646,
similarly suggest the use of fluorouridine monophosphate to be activated
by the enzyme
alkaline phosphatase conjugated to an antibody that binds to a
tumor cell surface antigen.

. . .
in fact more
than 100 fold less toxic than melphelan to particular cell lines in
culture, pretreatment of cells
with an antibody-PVA conjugate failed to enhance the toxicity
of the prodrag because PVA
hydrolyzed the phenoxyacetamide bond of the prodrug too slowly to
generate. . .

Catalyfic Proteins
a. E
.n=es
The prior art discloses the use of non-mammalian enzymes
conjugated to targeting antibodies
in order to activate the prodrug selectively at tumor sites (e.g.,
carboxypeptidases described in
Bagshawe, et al., Patent Application. . .

. . .
are administered;
neuraminidase removes the sialic acid residue at the terminus of
oligosaccharides on
glycoproteins (important components of erythrocyte membranes, for
example), exposing
galactose residues which mark such glycoproteins for rapid
degradation in the liver. Due
consideration of the situation in vivo is necessary for. . .

. . .
whose electrostatic and
shape characteristics can be mimicked by a phosphonate structure.
Immunization of a mouse
with a nitrophenyl phosphonate ester hapten-protein conjugate
led to the isolation of
monoclonal antibodies with hydrolytic activity on methyl-p-nitrophenyl
carbonate (Jacobs, et
al., J. Am. Chem. Soc. 109 (1987):2174-2176).. . .

. . .
could cleave an aryl carboxamide (Janda, et
al., Science 241 (1988):1188-1191). Another scheme for production of
peptidases utilized a
metal complex cofactor linked to a peptide (Iverson, et al.,
Science 243 (1989):1184-1188).

The term moiety as used herein with reference to immunoconjugates means
the whole
antibody, enzyme or targeting protein, or active
fragment thereof.

(i) a moiety capable of binding to an epitope of a specific cell
population, and
(ii) a catalytic antibody moiety or enzyme moiety
capable of activating said novel
prodrug of the subject invention.

(i) a moiety capable of binding to an epitope of a specific cell
population, and
(H) a catalytic antibody moiety or enzyme moiety

capable, of activating a novel
prodrug of the subject invention;
(b) permitting said immunoconjugate to become localized at said cell
population;. . .
. . .
2;
VL antibody I-S-VH antibody 1-S-VL antibody 2-S-VH antibody 2;
VL antibody I-S-VH antibody I-S-VH antibody 2-S-VL antibody 2;
wherein -S- is a linker sequence; and
(ii) isolating said bispecific antibody.
. . .
1-S-VL antibody 2,
(iii) combinin the products of steps (i) and (ii), and
9 I
(iv) isolating said bispecific antibody,
wherein -S- is a linker sequence.
. . .
antibody 2-S-VL antibody 1,
(M) combining the products of steps (i) and (ii), and
(iv) isolating said bispecific antibody,
wherein -S- is a linker sequence.

Figure 11 (Sheet 31/87) shows the site specific activation of
galactosyl-AraC prodrug
on CEA antigen positive cells.

Figure 12 (Sheet 32/87) shows the activity of galactosyl-AraC prodrug on
CEA antigen
negative cells.

Figure 18 (Sheet 38/87) shows the comparison of 5' fluorouridine and
galactosyl-5'
fluorouridine prodrug on CEA antigen negative Colo cells.

Figure 19 (Sheet 39/87) shows the site specific activation of
Tfluorouridine prodrug
on CEA antigen positive Lovo cells.

Figure 20 (Sheet 40/87) shows the, activity of 5'Ruorouridine prodrug on
CEA antigen
negative Colo cells.
. . .
in or near tumors by prior administration of tumor-selective
agents such as receptor-binding ligands, analogs which bind to tumor
associated'enzymes,
and antibodies conjugated to or otherwise physically connected
to a protein catalyst
which converts the prodrugs to active cytotoxic agents. The catalytic
protein is. . .

Catalytic antibodies, as well as enzymes, catalyze
chemical reactions by lowering the
activation energy required to form the short-lived, unstable transition
state. Catalytic
antibodies which] stabilize or enhance. . .
. . .
used as haptens for eliciting antibodies with catalytic
activity toward prodrugs of the invention. As such, their structure
generally includes a linker
arm for attachment to a protein carrier. Thus, the moiety of the hapten
corresponding to the
drug in the prodrug is typically an analog of the original drug,
differing in the presence of a
covalently-attached linker arm terminating in a group can be

attached to a prote . In
some embodiments of the invention, the linker arm is attached
to the moiety of the hapten
corresponding to the prodrug substituent (e.g., the substituted benzoate
portion of an. . .

The catalytic proteins of the invention are conjugated to, or
otherwise physically associated
with, a tumor-selective antibody, antibody fragment, or binding protein
or analogs to tumor-
associated proteins or tumor-selective. . .
. .
hydrolyzes acetals (or ortho esters) to aldehydes (or acids)
D. Glycosidase - cleaves sugar substittients attached to drugs via a
glycoside linkage.

Hexopyranose conjugated to cl]ug hydroxyl group via the
anorneric position of the
sugar.

2. Hexofuranose conjugated to drug hydroxyl group via the
anorneric position of the
sugar.
. .
in the structure of X. Typically, however, X' will be very similar to X,
generally
differing in that X' contains a linker arm for joining the
transition-state analog to a carrier
protein such as bovine serum albumin (BSA) or keyhole limpet hernocyanin
(KLH). . .

R2'
R3 R
RV
wherein X is an analog of X of compound AI a, and Y is optionally
linked to a
carrier protein,
B is 0, S, NH, or CH2,
D is P(O)OH, SO2, CHOH or SO (with any stereochemistry), if D is CHOH
then B
is CH2, and
RI', R2'q R3'q R4' and RY are the same or different, are optionally
linked to a carrier
protein and are H, alkyl with I - I 0 carbon atoms, alkoxy with I - I 0.
. .

R6'
RT
R8' D%s]'B0000 X
wherein Xis an analog of X of compound A2a, and X'is optionally
linked to carrier
protein,
B is 0, S, NH, or CH2,
D'is P(O), COH (with any stereochenuistry), if D'is COH then B and Yare.
. . ester groups, ether groups, -CO-,
cyano, epoxide groups and heteroatoms,
R6'j R7'9 R8', and R9' are the same or different, are optionally
linked to the carrier
protein and are H, alkyl with I - I 0 carbon atoms, alkoxy with I - I 0.
. .

Rio'
R11'
R12

wherein Yis an analog of X of Ma, and Yis optionally linked to carrier protein,
B is 0. S, NH, or CH2.
. . .
D is P(O)OH, SO2, CHOH or SO with any stereochemistry, if D is CHOH then B is
CH2, and
RIO'-12'which are optionally linked to the carrier protein, are the same or different but
at least two of them are not H, and are H. . .

6.]_ Y
I
D' x
R13
4
wherein X' is an analog of compound Ma, and X' is optionally linked to a carrier
protein,
B is 0, S, NH, or CH2,
D'is P(O), COH with any stereochemistry, if D'is COH, then B and. . .

R 16'
R17 R15'
R18 a%*,
wherein X' is an analog of X of compound B 1 a, and X' is optionally linked to the
carrierprotein,
B is 0, S, NH, or CH2,
D is P(O)OH, SO2. CHOH or SO with any stereochemistry, if D is CHOH then B is
CH2, and
R15', R16', R17% R18' and R19' are the same or different, are optionally linked to the
carrier protein, and are H, alkyl with 1-10 carbon atoms, alkoxy with 1-10 carbon atoms,
monocyclic aromatic, alkene with 1-10. . .

R20'
Ft21
X
R2Z D%,%B000e
wherein X is an analog of the drug XNH2 of compound B2a, and X is optionally
linked to a carrier protein,
B is 0, S, NH, or CH2,
D'is P(O), COH with any stereochemistry, if D'is COH then B. . .
substituents that are phenyl, alkyl, or alkyl with
heteroatoms, and;
R20% R21'.) R22'. and R23' are the same or different, are optionally linked to the carrier
protein and are H, alkyl with 1-10 carbon atoms, alkoxy with 1-10 carbon atoms, monocyclic
aromatic, alkene with 1-10. . .

wherein X is an analog of X of compound B3a, and X' is optionally linked to the
carrier protein,
B is 0, S, NH, or CH2, and
D is HP(O)OH, CH20H, P(O)(OH)2, or S03H, if D is CH20H. . .

R24!
Fi25' ell]x
R

wherein X is an analog of X of compound B4a, and X is optionally linked to the
carrier protein,
B is 0, S, NH, or CH2,
D is P(O)OH, SO2, CHOH or SO with any stereochen-dstry, if D is CHOH then B is
CH2, and
R24'-26'which are optionally linked to a carrier protein, are the same or different and
are H, alkyl with 2 to 22 carbon atoms, alkyl with. . .

J Y
R27 *']4%B000e
wherein X' is an analog of X of compound B5a, and X' is optionally linked to the
carrier protein,
B is 0, S, NH, or CH2,
D'is P(O), COH with any stereochemistry, if D'is COH then B and. . .
heteroatoms with 1-9
atoms in a linear configuration which have substituents that are phenyl, alkyl, or alkyl with
heteroatoms, and
R27-28'which are optionally linked to the carrier protein are the -same or different and
are H, alkyl with 2 to 22 carbon atoms, alkyl with. . .

In all strategies, the structure of the substituents will depend on the drug (occupying R)
conjugation to an immunogenic carrier protein including but not limited to KLH or BSA
(through R, R', or R) and the structure. . .
.
at least one of R30' and R3 F is an analog of X of compound B6a, and said
analog is optionally linked to a carrier protein,
D ... is S02, SO or CHOH with any stereochemistry, if D... is CHOH then
Eis CH,
Zis. . . heterocyclic or phenyl
substitution (optionally substituted on the heterocyclic or phenyl group), and
R29'is optionally S03H or S04H, and
R29v -3T are optionally linked to a carrier protein.

R 0
Fi36' 00000 X
B
R351 2)n
Al
wherein X'is an analog of X of compound B6c, and is optionally linked to carrier
protein,
B is 0, S, NH, or CH2,
R34]1 R35% R36'. and R37' are the same or different and are H,. . .

0
(CH2)n 000
k-]E' B
F14,V
wherein X'is an analog of X of compound B6e, and Xis optionally linked to carrier
protein,
B is 0, S, NH, or CH2,
n is an interger from 0 to 4,
R43' and R44' are the same. . .

Q'] X
wherein Q'is 0, S, NH, or CH2,
X is an analog of X of compound Cla, and X is optionally linked
to a carrier
protein,
B'is NE or CH2, if B'is NH, then Q'is CH2, and
R45' and R46' are the, same or different and are H, alkyl unsubstituted,
alkyl
substituted with halogens, heteroatoms, phosphonate, sulfonate,
carboxylate,
alkylanu-nonium, alkene, or monocyclic aromatic, and are optionally
linked to a carrier
protein.

different and are H, alkyl unsubstituted, alkyl
substituted with halogens, heteroatorns, phosphonate, sulfonate,
carboxylate,
alkylammonium, alkene, or monocyclic aromatic, and are optionally
linked to a carrier
protein.

Q'is 0, S, NH, or CH2,
wherein X' is an analog of X of compound Cl a, and X' is optionally
linked to a
carrier protein, and
R45' and R46' are the same or different and are H, alkyl unsubstituted,
alkyl
substituted with halogens, heteroatoms, phosphon-ate, sulfonate,
carboxylate,
alkylammonium, alkene, or monocyclic aromatic, and are optionally
linked to a carrier
protein.

different and are H, alkyl unsubstituted, alkyl
substituted with halogens, heteroatoms, phosphonate, sulfonate,
carboxylate,
alkylammonium, alkene, or monocyclic aromatic, and are optionally
linked to a carrier
protein.

R45 is H, aminocarboxy, alkyl unsubstituted, alkyl substituted with
halogens, heteroatoms, phosphonate, sulfonate, carboxylate,
alkylammonium, alkene or
monocyclic aromatic, and are optionally linked to a carrier
protein.

When E which is linked to R45, is N, it is preferred that the
E-R45, linkage forms an
amide substituted with an amine moiety.

47
R -NH
wherein X'is an analog of X of compound C2a, and which is optionally
linked,to a
carrier protein,
Q'is 0, CH2, S, or NH, and
R471 and R48' are the same or different and are H, alkyl unsubstituted,
alkyl
substituted with halogens, heteroatoms, phosphonate, sulfonate,
carboxylate,
alkylammonium, alkene, or monocyclic aromatic, and are optionally
linked to a carrier
protein.

. . .
different and are H, alkyl unsubstituted,
alkyl substituted with halogens, heteroatorns, phosphonate, sulfonate,
carboxylate,
alkylammonium, alkene, or monocyclic aromatic, and are optionally
linked to a carrier
protein.

47
NH
wherein X'is an analog of X of compound C2a, and which is optionally
linked to a
carrier protein,
Q'is CH2, and
R47' and R48' are the same or different and are H, alkyl unsubstituted,
alkyl
substituted with halogens, heteroatoms, phosphonate, sulfonate,
caTboxylate,
alkylammonium, alkene, or monocyclic aromatic, and are optionally
linked to a carrier
protein.
. . .
different and are H, alkyl unsubstituted, alkyl
substituted with halogens, heteroatoms, phosphonate, sulfonate,
carboxylate,
alkylammonium, alkene, or monocyclic aromatic, and are optionally
linked to a carrier
protein.
. . .
with halogens, heteroatoms, phosphonate, sulfonate, carboxylate or alkyl
ester or
alkyl amide, hydroxyl, alkylammomum, o, alkene, or monocyclic aromatic,
and are
optionally linked to a carrier protein.

RSD'
- B1
Rs' NH
wherein Q'is 0, S, NH or CH2,
Yis an analog of X of compound C3a, and Xis optionally linked
to a carrier
protein,
B'is NH or CH2, if Bis NH, then Q'is CH2, and
R50' and R51' are the same or different. . . with halogens,
heteroatoms, phosphonate, sulfonate, carboxylate or alkyl ester or
alkyl an-dde, hydroxyl, alkylammonium, amino, alkene, or monocyclic
aromatic, and are
optionally linked to a carrier protein.
. . .
R50'
R51' x
wherein Q'is 0, S, NH or CH2,
X' is an analog of X of compound C3a, and X' is optionally
linked to a carrier
protein, and
R50' and R51' are the same or different and are H, alkyl unsubstituted,
alkyl
substituted with halogens, heteroatoms, phosphonate, sulfonate,
carboxylate or alkyl ester or
alkyl arnide, hydroxyl, alkylammonium, an-dno, alkene, or monocyclic
aromatic, and are
optionally linked to a carrier protein.
. . .
with halogens, heteroatoms, phosphonate, sulfonate, carboxylate or alkyl

ester or
alkyl amide, hydroxyl, alkylammonium, amino, alkene, or monocyclic aromatic, and are
optionally linked to a carrier protein.

. . .

a,- X
wherein Q'is 0, S, NH or CH2,
X' is an analog of X of compound Of, and X is optionally linked to a carrier
protein,
Wis NH or CH2, if B'is NH, then Q'is CH2, and
G' is a radical of the diol G(OH)2,. . . cycloalkyldiol or ortho-phenyldiol, and G' is optionally substituted with halogens, heteroatoms, phosphonate,
sulfonate, carboxylate, alkylarnmonium, alkene, or monocyclic aromatic, and is optionally
linked to a carrier protein.

. . .

cycloalkyldiol or ortho-
phenyldiol, and G' is optionally substituted with halogens, heteroatoms, phosphonate,
sulfonate, carboxylate, alkylammonium, alkene, or monocyclic aromatic, and is optionally
linked to a carrier protein.

QI]X
wherein Q'is 0, S, NH or CH2,
X'is an analog of X of compound Of, which is optionally linked to a carrier protein,
and
G' is a radical of the diol G(OH)2, G(OH)2 is a sugar, cycloalkyldiol or ortho-
phenyldiol, and G' is optionally substituted with halogens, heteroatoms, phosphonate,
sulfonate, carboxylate, alkylammonium, alkene, or monocyclic aromatic, and are optionally
linked to a carrier protein.

. . .

cycloalkyldiol or ortho-
phenyldiol, and GI is optionally substituted with halogens, heteroatoms, phosphonate,
sulfonate, carboxylate, alkylammonium, alkene, or monocyclic aromatic, and are optionally
linked to a carrier protein.

. . .

with halogens, heteroatoms, phosphonate, sulfonate, carboxylate or alkyl ester or alkyl amide, hydroxyl, alkylammonium, amino, alkene, or monocyclic aromatic, and
are optionally linked to a carrier protein.

R52 t
X
NH
wherein X'is an analog of X of compound C4a, and which is optionally linked to a
carrier protein,
Q'is CH2 or NH, and
R52T and R531 are the same or different, and are H, alkyl unsubstituted, alkyl
substituted. . . with halogens, heteroatoms, phosphonate, sulfonate, carboxylate or alkyl ester or
alkyl amide, hydroxyl, alkylammonium, amino, alkene, or monocyclic aromatic, and are
optionally linked to a carrier protein.

. . .
with halogens, heteroatoms, phosphonate, sulfonate, carboxylate or alkyl ester or alkyl amide, hydroxyl, alkylammonium, amino, alkene, or monocyclic aromatic, and
are optionally linked to a carrier protein.

W
Q1- X
R NH
wherein X is an analog of X of compound C4a, and which is optionally linked to a
carrier protein,
Q is CH2, and
R52' and R53' are the same or different, and are H, alkyl unsubstituted, alkyl
substituted with halogens, heteroatorns, phosphonate, sulfonate, carboxylate or alkyl ester or
alkyl amide, hydroxyl, alkylammonium, amino, alkene, or monocyclic aromatic, and are
optionally linked to a carrier protein.

a,- X
N
wherein Yis an analog of X of compound C4, and which is optionally linked to a
carrier protein,
Q'is CH2 or NH, and
G' is a radical of the diol G(OH)2, G(OH)2 is a sugar, cycloalkyldiol or ortho-
phenyldiol, and G' is optionally substituted with halogens, heteroatoms, phosphonate,
sulfonate, carboxylate, alkylammonium, alkene, or monocyclic aromatic, and is optionally
linked to a carrier protein.
. . .
cycloalkyldiol or ortho-
phenyldiol, and G' is optionally substituted with halogens, heteroatoms, phosphonate,
sulfonate, carboxylate, alkylammonium, alkene, or monocyclic aromatic, and is optionally
linked to a carrier protein, and
R59' is H, alkyl unsubstituted, alkyl substituted with halogens, heteroatoms,
phosphonate, sulfonate, carboxylate or alkyl ester or alkyl amide, hydroxyl, alkylammonium,
amino, alkene, or monocyclic aromatic, and is optionally linked to a carrier protein.

G] Qv])C
NH
wherein Xis an analog of X of compound C4, and which is optionally linked to a
carrier protein,
Q'is CH2. and
G' is a radical of the diol G(OH)2, G(OH)2 is a sugar, cycloalkyldiol or ortho-
phenyldiol, and G' is optionally substituted with halogens, heteroatoms, phosphonate,
sulfonate, carboxylate, alkylammonium, alkene, or monocyclic aromatic, and is optionally
linked to a carrier protein.
. . .
cycloalkyldiol or ortho-
phenyldiol, and GI is optionally substituted with halogens, heteroatoms,

phosphonate,
sulfonate, carboxylate, alkylammonium, alkene, or monocyclic aromatic,
and is optionally
linked to a carrier protein.

. . .
the
nucleotide analog, in particular such prodrugs wherein said hexopyranose
or hexofuranose is
selected from the group consisting of glucose, glucosamine,
D-quinovopyranose, galactose,
galactosamine, L-fucopyranose, L-rhamnopyranose, D-glucopyranuronic
acid, D-
galactopyranuronic acid, D-mannopyranuronic acid, or D-iodopyranuronic
acid.

. . .
a monosaccharide hexopyranose
or hexofuranose which is a structural analog of a sugar selected from
the group consisting of
glucose, glucosamine, D-quinovopyranose, galactose,
galactosamine, L-galactopyranuronic
acid, D-mannopyranuronic acid, or D-iodopyranuronic acid.

A novel coupling reaction to make P-glycosylated nucleosides
of the invention from
hexopyranoses and nucleosides is the direct reaction of the
peracetylated hexoses and. . .

The coupling reaction can be accomplished also by activation
of the anomeric position by
conversion to SPh, F or imidate groups and subsequent. . .

. . .
OH CH20H H
Glucosan-dne H OH H OH H NH2 CH20H H
D - Quinovopyranose H OH H OH H OH CH3 H
Galactose OH H H OH H OH CH20H H
Galactosan-dne OH H H OH H NH2 CH20H H
L-Fucopyranose OH H H OH
L-. . . manopyranuronic acid H OH H OH OH H COOH H
lodopyranuronic acid H OH H OH H OH H COOH
The coupling reaction of hexofuranoses at their anomeric
position to the nucleoside 5'
position to make furanosylated nucleosides can be accomplished by the.
. .

Coupling of hexofuranoses to nucleosides will make a mixture
of anomers, because of the
ring size.

D. Prodru2 Activation By Glycosidase Reaction
I . Hexopyranose conjugated to drug hydroxyl group via the
anomeric
position of the sugar
2 . Hexofuranose conjugated to drug hydroxyl group via the
anomeric
position of the sugar.

Q is 0 or NH, and
V is a hexopyranose or hexofuranose conjugated to QX via the
anomeric position of
the sugar with optional alpha or beta configuration.

Advantageously V is Glucose, Glucosamine, D-Quinovopyranose,
Galactose,
Galactosamine, L-Fucopyranose, L- Rhamnopyranose, D-Glucopyranuronic

acid, D-
Galactopyranuronic acid, D-manopyranuronic acid, or D-Iodopyranuronic acid.

The synthesis of the haptens is accomplished by the coupling reaction of the appropriate
lactam and the corresponding 5 amino nucleoside in the presence of triethyloxonium
tetrafluoroborate in methylene chloride as the. . .

A hapten (amidine TS analog) for galactose or equivalent sugar for the cleavage of the
glycosidic bond to liberate drug, said hapten having the formula.

N
I \\Ql]x
M, le
wherein X'is an analog of X of compound Dla, and which is optionally linktad to a
carrier protein,
Q'is NH, and
M' is a 1,4-diradical of a n-pentane where Cl, C2, C3 and C5 are optionally
substituted with OH, and Mis optionally linked to a carrier protein.

. . .
NH
ml
M' is a 1,4-diradical of a n-pentane where Cl, C2, C3 and C5 are optionally
substituted with OH, and M'is optionally linked to a carrier protein.

NH
M Qv] X
wherein X' is an analog of X of compound DI a, and which is optionally linked to a
carrier protein,
Q'is CH2, and
M' is a 1,4-diradical of a n-pentane where Cl, C2, C3 and C5 are optionally
substituted with OH, and Mis optionally linked to a carrier protein.

. . .
attached to certain cyclophosphan-iide metabolites (e.g., 4-hydroxycyclophosphamide or aldophosphamide) prevents their enzymatic and chemical
breakdown to cytotoxic products. An appropriate protein catalyst, conjugated to a tumor-
selective reagent, is administered prior to the prodrug-, the catalyst thereupon produces active
alkylating species in the vicinity of. . .

. . .
0
CARRIER PROTEIN]N N 02H
\]J
Prodrugs of Other AntineWlastic Agents
Prodrugs of a wide variety of antineoplastic agents are prepared by their conjugation to
prodrug substituents of the invention. Ester or glycosyl substituents of the invention are
appropriate for drugs with hydroxyl groups; amide substituents. . .

. . .
hydrolyzes acetals (or ortho esters) to aldehydes

Alkenyl-glycerophosphocholine hydrolase (E.C. 3 2.2)
Cellulase (E.C. 3 1.4)
Oligo-1, 6-glucosidase (E.C. 3 1.10)
Lysozyme (E.C. 3 1.17)
P-D-Glucuronidase (E.C. 3 1.31)
D. Glycosidase - cleaves sugar substituents attached to drugs via an ether linkage
Examples include beta-galactosidases, beta-glucosidases, inulases, alpha-L-
arabinofuranosidases, agarases, and isomerases. Specific examples include.
. . .
glycoprotein Goldrosen, M, et al.,
hepatic metastasis Cancer Research 50
(1990) 7978
It
Adenocarciriorna GI tract and other F023C5 Camino-embryonic SiccardL A., Cancer
tissues antigen (CEA) Research 50 2-
(1990):899s-903s
Carcinoma Head/Neck and E48 Peptide epitope within Gerretsen, M., et al.,
Vulva 22kD surface antigen British Journal of Cancer
63 (1991):37-44
Carcinoma Larynx, pharynx CEA Kairemo, K., et a., A=
and parotid gland Ongoloica 29
(1990):539-543
Carcinoma Liver NP-4 CEA Wang, Z., et al., Cancer
Research 0
(1990):869s-872s
Carcinoma Breast CEA Kairemo, K., et al., A=
Oncologic 29
(1990):533-538
Carcinoma Bladder BW 431/26 CEA Boekinann, W., et al.,
British Journal of Can
62 (1990):81-84
Carcinoma Ovary HWGI Milk fat blobule glyco- Hird, V., et al., British
protein (>200M). . .
. . .
of Cancer 63 (1991):37-44). Successful imaging has
been possible even in patients with significant serum concentration of antigens shed from the
tumor (CEA, Boeckmann, W., et al., British Journal of Cancer
62 (1990):81-84).
. . .
Chaudhary, et al., Proc. Natl. Acad. Sci. USA 84 (1987):4538-4542; Kondo J., et al.,
Biol. Chem. 263 (1988):9470-9475). Generation of analogous
fusions using the growth
factors interleukin 6, interleukin 2, transforming growth factor alpha, and others are made by
linking enzymes or abzymes using the methods described in the above references. The
incorporation of catalytic antibodies into these is done via the fusion of these growth factors
to the end of antibody single chain gene constructs (Patent Application WO 88/01649) or
alternatively the growth factors. . .

The use of human CD4-Pseudomonas exotoxin fusion has proved effective in the killing of
HIV infected cells. The use of such a binding activity from CD4 linked to an enzyme or
catalytic antibody allows the use of prodrug therapy directed at

treatment of AIDS. The CD4
binds to the gp120 expressed on FHVI infected cells. The converse of such a construct
makes use of gp120-enzyme (or catalytic antibody) fusion to develop an immunosuppression
reagent system (Moore et al., Science 250, (1990):1139). Other g species which are
useable in the subject invention. . .

Production of Bi]pecific Prg=iU
a. Production of Bi4ecific Prgteins by Chen-dcal Linkage of Enzymes or Catalytic
Antibodies to Targeting Proteins
7be enzymes of this invention can be covalently bound to the targeting proteins of this
invention by techniques well known in the art such as the use of the heterobifunctional cross-
linking reagents SPDP (N-succinimidyl-3(2-
pyridyldithio)proprionate) or SMCC
(succinimidyl 4-(N-maleimidomethyl) cyclohexane- I -carboxylate [see., e.g., Thorpe, P. E.,
et al., The Preparation and Cytotoxic Properties of Antibody-Toxin Conjugates.
Immunological Rev., 62 (1982):119-58; Lambert, J. M., et al.,.supra at p. 12038;
Rowland,G. F., et al., supra, at pp. 183-84 and Gallego,. . .

b. Production of Bispecific Proteins by Recombinant DNA
Fusion proteins comprising at least the antigen binding region of the targeting protein of the
invention linked to at least a functionally active portion of an enzyme or catalytic antibody of
the invention can be constructed using recombinant DNA techniques well known in the art
[see, e.g., Neuberger, M. S., et al., Nature 312, (1984):604-608]. These fusion proteins act
in essentially the same manner as the antibody-enzyme conjugates described herein.

. . .
required enzymatic activity follows these basic procedures. To achieve the
optimized level of enzyme activity, manipulation of the sequences between the antibody and
enzyme may be needed. Addition of linker sequences
and/or alteration of the fusion site may
be needed for this optimization. In addition, to the advantage of a defined antibody-enzyme
reagent, the reduced size possible by the removal of the CH2 CH3, and the CH4 in the case if
IgE and IgM. . .

. . .
Methods for generation of bispecific antibodies consist of chemical methods of separation and
recombination of the antibody chains or by the fusion of the two hybridomas to generate so
called quadromas. These methods are effective but are prone to generate rriixed species and
require. . .

. . .
chain antibodies, in which the variable
(V) region of the two antibody chains are combined into a single molecule using a linker
sequence (Patent Application WO 88/01649, Ladner and Bird). This combination of V

regions results in expression of a protein which has one. . . of the V regions at the amino it terminus and the other V region attached at its COOH terminus via the linker to its an-dno terminus. This head to tail, head to tail linkage of V regions has been described with both V light chain - V heavy chain and V heavy chain - V. . .

The construct would consist of. the V Heavy chain region (VH) linked to the V Light chain region (VL); specific for the tumor cell or antigen via the linkers described for single chain antibodies (Vijay, et al., Nature 339 (1989):394-397; Patent Application WO 88/01649, Ladner and Bird); these sequences are linked directly to the catalytic antibody VL which can also follow VL-VH-VH-VL or VL-VH-VL-VH or VH-VL-VH-VL sequences. The linker sequences used in these constructions are those described above for single chain antibody construction. This combination allows the expression of a. . . species based on similar construction describes a previously unknown molecule as follows. The VH region specific for the tumor or antigen linked directly to the VL region of the catalytic antibody; this molecule is advantageously expressed separately or together with the other construct of VL specific for the tumor or antigen linked directly to the VH of the catalytic antibody. The expression products of these two molecules together, or by post expression mixing, associate. . . a lower molecular weight. The use of single domain binding proteins is also valuable to explore in the form of direct fusions to enzymes or catalytic antibodies (Patent Application WO 90/05144, Winter).

. . .

antibody expression library. In this system, rather than having the VH and VL genes as separate transcription units, they are covalently linked by a short peptide to produce a single chain antibody as defined by Bird, E., et al., Science 242 (1988) 426). Using. . .

. . .

use in this selection would include those haptens described herein. These antigens used for the raising of antibodies can also be coupled indirectly to a solid phase, such as epoxy activated Sepharose CL-6B, via coupling to a protein carrier. The choice of carrier protein is made such that the protein used for immunization would not be. . .

. . .

the cell wall of a P-lactamase enzyme-deficient E. coli causing death or impaired growth. In addition, these substituents optionally are used in coupling a carrier protein (KLH or BSA) during immunization.

. . .

portion may abolish or diminish the antibiotic action of aztreonam on E. coli. Screening with aztreonam rather than with the larger aztreonam-drug conjugate is acceptable because the antibodies are raised to a hapten that included the drug or an analog thereof and mutant antibodies. . .

Detection of prodrug activation is carried out by colorometric or fluorometric determination of

the generation of galactose, which accompanies prodrug activation.

One of a number of possible galactose detection methods is employed. Some sensitive and specific detection methods follow-

I . Radiolabelfing of free galactose with N_E:]ph]ha

a) Galactokinase (E.C. 2 1.6) is commercially available (Sigma Chemical Co., St.

Louis, USA) and catalyzes the following reaction;

galactose + AT? -->P- galactose- I -phosphate + ADP

If the ATP (adenosine triphosphate) used has 32p in the gamma phosphate position, free

galactose generated by catalytic antibodies becomes radioactively-labelled. Labelled

galactose-1 -phosphate is separated from the other constituents in the reaction mixture by thin layer chromatography (TLC) or high performance liquid chromatography.

In this type of detection method, galactose is non-catalytically reacted with commercially available (from, for example, Molecular Probes, Inc., Eugene, OR) aldehyde-reactive reagents to yield a colored or fluorescent derivative. The product of the reaction with

galactose is isolated by HPLC or by TLC and detected by absorbance or by fluorescence by standard means.

Detection of galactose with polor-g]nerating Mecific enMms.

a) One enzyme that can be used to detect galactose is galactose dehydrogenase (E.C.

1 I A8) (Sigma Chemical Company, St. LDuis, MO, USA) which catalyzes the following oxidation-reduction reaction;

galactose + NAD+ (no color) --*] galactonate + NADH (color) + H+

The oxidation of galactose is accompanied by the reduction of nicotinamide adenine dinucleotide (NAD+). The reduced form of NAD+, NADH, is colored and its appearance.

We

b) An alternative enzyme that is useful in detecting galactose is galactose oxidase, (E.C. 1 3.9) which, used in combination with peroxidase and o-tolidine, will cause a color change in response to the presence of free galactose generated by a catalytic antibody. The coupled reactions are as follows. The first reaction is catalyzed by galactose oxidase and the second by peroxidase, both available from Sigma Chem. Company;

1. galactose + 0,2 --a galactonate + H202

lv]

2. H202 + o-tolidine H20 + colored product

The colored product generated can be measured spectrophotometrically.

A third method for in vitro detection of hydrolysis of aromatic ester nucleoside analogs is to

use an enzyme-linked assay. One inexpensive
commercially-available enzyme (Sigma
Chemical Company, St. Louis, MO) that could be used for this purpose is
thymidine
phosphorylase (E.C. 2 2.4). This enzyme converts the substrates
thymidine and
orthophosphatetotheproductsthyn-iineand2-deoxy]-D-ribose-l-phosphate.
Ratherthanthe
prodrug being screened here, a conjugate of the inactivating
ester with thymidine will be used
(the same types of compounds that will be used in biological screening.
. .

c) A third possible enzyme-coupled detection method employs
both alcohol oxidase
(E.C. 1 3.13) and peroxidase (E.C. 1 1.7). Alcohol oxidase can convert
an
aldehyde to a. . .

. . .
wherein a marnalian host is
treated in a phan-naceutically acceptable manner with a pharmaceutically
effective amount of a
targeting protein catalytic protein conjugate or
conjugates or bispecific antibody or antibodies
and a pharmaceutically effective amount of a prodrug or prodrugs. The
combination and
methods of this invention. . .

. . .
target and localize at the tumor site. Such sufficient time may range
from
4 hours to one week depending upon the conjugate used. The
period of time between the end
of administration of the immunoconjugate and the beginning of
administration of prodrug
varies depending. . .

. . .
or infusible solutions. The preferred form depends
upon the mode of administration and the therapeutic application. For
example, oral
administration of the antibody-enzyme
conjugate or bispecific antibody may be disfavored
because the conjugate proteins tend to be degraded in the
stomach if taken orally, e.g., in
tablet form.

. . .
Another embodiment of this invention of this invention provides a method
of combination
chemotherapy using several prodrugs and only a single antibody
-enzyme conjugate.

. . .
of prodrugs are used that are all substrates for the
same enzyme or catalytic antibody in an immunoconjugate. Thus, a
particular antibody-
enzyme conjugate or bispecific antibody converts a
number of prodrugs into cytotoxic form,
resulting in increased antitumor activity at the tumor site.

. . .
and one wants to be certain that sufficient enzyme is
targeted to the tumor site. The use of a number of conjugates
bearing different antigenic
specificities for the tumor increases the likelihood of obtaining
sufficient enzyme at the tumor
site for conversion of a. . .

. . .

(2 x 20 mL) in water (20
mL). The organic layer was separated, dried, concentrated, and flash chromatoghaphed to
afford the coupled compound as an oily material (0.66 g, 95 %,
Rf, 0.46, silica, methylene
chloride, methanol, hexane, 80, 1, 19).

. . .

were protected to give iodide 3c. 3-
Butyne-l-ol was transformed in four steps to alkyne 3d. Alkyne 3d and iodide 3c are
coupled using a Pd(11) catalyst to give nucleoside analog 3e
(Robins, J. J., et al., J. Org.

. . .

2a, which is reacted with alcohol M to give diester 3g. Reduction and basic hydrolysis gives hapten 4, which can be linked to a
carrier protein via the primary
amino group.

. . .

dihydroxy compound 2c. Compound 2c, on treatment with PCI5 in
chloroform at 5C] C afforded the phosphochloridate 2d. Compound M was coupled with
phosphochloridate 2d in methylene chloride in the presence of DMAP to afford coupled
compound 3h. Compound 3h was hydrogenated using Pd-C in ethyl acetate to afford the
debenzylated compound which on treatment with aqueous ammonia. . .

Subsequent coupling with P-galactose pentacetate in
the presence of trimethylsilyl
trifluoromethanesulfonate in acetonitrile yielded the partially protected compound 17.

Couph
.:ng reaction-, BLeparation of Co=ound 17
To a solution of galactose penta acetate (1.17 g, 3 mmol) and compound 16 (2 mmol) in dry
acetonitrile (5 mL), a solution of trimethylsilyl. . .

. . .

21. Deprotection
of the silyl group was achieved using p toluene sulphonic acid at O' C, and the resultant
product 22 was coupled with P-galactose pentacetate
in the presence of trimethylsilyl
trifluoromethanesulfonate in acetonitrile to give the fully protected compound 23. Complete
deprotection with ammonia in methanol. . .

13C HNMR (CDC13): 170.34, 170.08t 157.56, 149.55, 139.17, 86.61, 83.72, 73.21
71.48] 61,65, 20.65, 20
Preparation of coupling compound 23: Coupling
reaction between galactose penta acetate
and compound 22 was accomplished by the method as mentioned above to give coupling
product 23 (Rf, 0.20, 1:1 EtOAC: Hexane, 59%).

. . .

an amine which is then deprotected with
sodium methoxide in methanol to give the aminonucleoside 25. This aminonucleoside is
used in subsequent coupling reactions to give the amidine compound 30b (R=5-
fluorouridine).

. . .

Inversion of the

secondary hydroxy group using the Mitsunobu reaction procedure gives the galactolactam
29b derivative. Activation with Meerweins reagent and subsequent coupling with the amino
nucleoside 25 (Example 5a) followed by desilylation with fluoride gives the final amidine
compound 30b (R= 5-fluorouridine).

Preparation of the galactose-P fluorouridine amidine using 5-fluorouridine starts with
commercially available glucopyranose 31. Treatment with 2,2 dimethoxypropane in acetone
in the presence of catalytic amount. . . dimethylchlorosilane yields the glucolactarn compound 29a. The secondary hydroxy
group is inverted using the Mitsunobu reaction procedure and the subsequent activation and
coupling of the amide is accomplished using Meerweins reagent and the arninonucleside 25
(Example 5a) respectively. Final deprotection using fluoride yields the. . .
enol phosphonate 56. Deprotection and reaction with
phosphorus oxychloride, followed by N-trifluoroacetylpiperazine and then ammonia gives the
hapten 57, which can be linked to a carrier protein through
the piperazine ring.

To test if the prodrug could be activated by P-glact-osidase, the enzyme was conjugated to an
antibody that Was directed a
,gainst Carcino Embryonic Antigen (CEA) a specific tumor antigen
on the surface of the LoVo culture cells. The Colo 320 DM cells lack this surface antigen and
were used as controls. The P-galactosidase conjugated antibody was added to the cultures
and allowed to bind the antigen. The prodrug, at different concentrations, was then added to
the. . .

Figure 11 displays the results of this experiment which shows that the prodrug can be
activated by the antibody-enzymt conjugate. By comparing Figure I I with Figure 12, it is
clear that the prodrug was not only activated by the conjugated antibody but also that the
LoVo cells, carrying the CEA tumor marker are specifically killed when compared to the
cultures where BSA was added with prodrug. The above results show that. . .

To assess the ability of surface-bound conjugate to generate cytotoxic levels of active drug,
the rate of product formation was measured using ONPG as a substrate. Conjugate
specifically bound to LoVo cells was found to generate 1.2 x 107 molecules of
product/min/cell. In our particular assay format, this rate. . . .
be activated in vitro to produce levels of the drug similar to the pure drug itself. Thus, the
addition of the galactose moiety onto the drug reduces the toxicity substantially and to a level
that makes it an excellent candidate for a prodrug.

Galactosyl fluorouTidine has been tested for targeted activation by P-galactosidase
conjugated to an antibody with the same CEA antigen tumor surface specificity as was done
with the galAraC prodrug. The antibody was allowed to react with antigen-carrying cells
(LoVo) and control cells without the CEA antigen marker. The prodrug was then added to
different cultures at different concentrations.

Alcohol 74 undergoes a conjugate addition to ethynylsulfonate 75 to give the enol ether 76.

. . .

azido, and alkynyl groups gives amino alcohol 79, which is N-acylated and deprotected to give compound 81, which can be linked to a carrier protein at the
primary aliphatic amino group.

However, the trifluoroacetate salt may be used for the reaction linking compound 81 at the
ary aliphatic amino group to the carrier protein.

. . .

tissues. Fluorouridine is, however, toxic to normal tissues, particularly bone marrow and gastrointestinal epithelium. Prodrugs of fluorouridine that are
activated by catalytic antibodies or enzymes targeted to tumor cells improve the therapeutic
index of fluorouridine substantially. It is preferred that the prodrugs not be activated by
endogenous. . .

. . .

C mice. This dose is far
higher than would be administered in a therapeutic scenario involving targeted activation of
fluorouridine prodrugs by anti-linked catalytic proteins.

. . .

mice to determine whether
aldophosphamide diethyl acetal would in fact be relatively non-toxic and therefore suitable for
targeted activation by an antibody-catalyst conjugate.

A linker moiety was first prepared, and then attached to the phosphorus of the hapten. The
nitrogen of glycm'e was protected as the. . . The carboxyl group was then activated as the N-hydroxysuccinin-dde ester, forming compound
114, which was reacted with excess piperazine to form the linker moiety, compound 115.

. . .

to mice to determine whether
aldophosphamide diethylacetal would in fact be relatively non-toxic, and therefore, suitable
for targeted activation by an antibody-catalyst conjugate.

Arch. Allergy A12121. Immunol. 94 (1991):11-20). The host immune response can be
substantially reduced by conjugation of foreign proteins to, for example, copolymers of D-
glutamic acid and D-lysine (D-GL), polyethylene glycols (PEG), monomethoxypolyethylene
glycols (mPEG), or polyvinyl alcohols (PVA) (Sehon, A. H., Suppression of the IgE
Antibody Responses with Tolerogenic Conjugates of Allergens

and Haptens, In Progress In
AllerZX, Vol. 32 (1982):161-202). In each case, a protein such as an antibody (Ab) is
modified with multiple molecules (n) of the conjugate; i.e. Ab(PEG)n. The suppression of
the immune response depends on an optimum value of n; if n is too small or. . .

M., Sehon, A. H., Synthesis, Isolation, and Characterization of Conjugates of Ovalbumin
with Monomethoxypolyethylene Glycol using Cyanuric Chloride as the Coupling Agent,
Anal. Biochem. 165 (1987):114-127). The optimal value of n can be determined without
undue experimentation by one skilled in the art. . .

Conjugation of a catalytic antibody or catalytic/tumor-binding bispecific antibody to
nonantigenic molecules can be carried out as follows (Jackson, C. and J. C., Charlton, J. L.,
ski, K., Lang, G. M., Sehon, A. H., Synthesis, Isolation, and Characterization of
Conjugates of Ovalburnin with Monomethoxypolyethylene Glycol using Cyanuric Chloride
as the Coupling Agent, Anal. Biochem. 165 (1987):114-127). The optimum value of n (see
above) is deterniined experimentally by one skilled in the art and the procedure can be varied
to achieve this degree of conjugation. Preferably the antibody is conjugated to mPEG,
although other conjugates may also provide the desired effea MPEG is preferred over PEG
because PEG has two terminal hydroxyl groups which may participate in undesirable intra-
and inter-molecular crosslinking of conjugates (Sehon, A. H., Suppression of Antibody
Responses by Chemically Modified Antigens, Int. Arch. Allergy Mpl. Immunol. 94
(1991):11-20). The type of mPEG,. . . = 20,000) may also be chosen without undue
experimentation. Additionally, the scale of the procedure is altered accordingly, depending
on how much conjugated antibody is available or required.

2. The mPEG intermediate is reacted in the correct proportions with the antibody to
form a conjugate with the desired value of n.

To obtain the antibody-mPEG conjugate (Ab(mPEG)) of varying n, different amounts of
mPEG intermediate is added to 40 mg Ab. which is dissolved in sodium tetraborate. . .

Following conjugation, the mixture is passed through a Sephadex G-25 column (2.5 x 40
cm) equilibrated with 25 mM Tris buffer, pH 8 The conjugates are finally purified on a
DEAE-Trisacryl column (5 x 40 cm) pre-equilibrated in 25 MM Tris, pH 8 The protein is
bound. . . 5-Fluorouridine. The transition state analogue, the phosphonate ester of dimethoxy benzoyl fluorouridine compound 155 is prepared as
described in Example 44. After conjugation of the phosphonate analogue to the carrier

protein, keyhole limpet hemocyanin it is used to immunize mice and to produce monoclonal
antibodies. . .
. . .
single chain antibody gene is then combined with a single chain gene for the catalytic
antibody isolated as described above. The linking of these two single chain genes is in the
form of the linkers already described for the combination of the single chains or other
sequences known to be involved with the linkage of antibody domains; specifically genes
coding for (ser-lys-ser-thr-ser)3, or hinge regions. These linked genes are then placed into an
expression vector; in this instance, the vector pRC/CMS from In Vitrogen Inc., or other
similar expression. . .

CLMEN. . . . A compound having the formula:
R2'
R3 R
R4
wherein X' is an analog of X of Claim 1, and Y is optionally linked to a carrier
protein,
B is 0, S, NH, or CH2,
D is P(O)OH, SO2, CHOH or SO, if D is CHOH then B is CH2, and
RI', RTI RTJ R4' and R5' are the same or different, are optionally linked to a carrier
protein and are H, alkyl with I - I 0 carbon atoms, alkoxy with I - I 0.
. .

11 A compound having the formula-
R6'
RT
Df,],]B00000)C
R9'
wherein X' is an analog of X of Claim 7, and X' is optionally linked to carrier
protein,
B is 0, S, NH, or CH2,
D'is P(O), COH, if D'is COH then B and Yare CH2,
Y' is 0,. . . groups, ether groups, -CO-,
cyano, epoxide groups and heteroatoms.
R6'q R7 t R8', and R9' are the same or different, are optionally linked to the carrier
protein and are H, alkyl with I - I 0 carbon atoms, alkoxy with I - I 0.
. .
. . .
A compound having the formula:
R10 t
RI' B*#,%)C
R
wherein X' is an analog of X of Claim 13, and X is optionally linked to carrier
protein,
B is 0, S, NH, or CH2,
D is P(O)OH, SO2, CHOH or SO, provided that if D is CHOH then B is CH2, and
RIO'-IT which are optionally linked to the carrier protein,
are the same or different but
at least two of them are not H, and are H. . .
. . .
20 A compound having the formula:

R13 M]
14,
wherein X' is an analog of X of Claim 17, and X is optionally linked to a carrier protein,
B is 0, S, NH, or CH2,
D'is P(O), COH, provided that if D'is COH, then B and Yare. . . heteroatorns with 1-9 atoms in a linear configuration which have substituents that are phenyl, alkyl, or alkyl with heteroatoms, and
R13'-14'which are optionally linked to a carrier protein are the same or different but at least two of them are not H, and are H. . .

24 A compound having the formula:
R's
R17 R15'
IR18 x
wherein Kis an analog of X of Claim 21, and X'is optionally linked to the carrier protein,
B is 0, S, NH, or CH2,
D is P(O)OH, SO2, CHOH or SO, if D is CHOH then B is CH2, and
R15'.) R16'] R17', R18'and R19' are the same or different, are optionally linked to the carrier protein, and are H, alkyl with I - I 0 carbon atoms, alkoxy with I - I 0. . .

28 A compound having the formula:
FP'
R21
X
B 00e
wherein X'is an analog of the drug XNH2 of Claim 25, and X'is optionally linked to a carrier protein,
B is 01 S, NH, or CH2,
D'is P(O), COH, provided that if D'is COH then B and Yare. . . have substituents that are phenyl, alkyl, or alkyl with heteroatoms, and
R20', R21', R=, and R23'are the same or different, are optionally linked to the carrier protein and are H, alkyl with 1-10 carbon atoms, alkoxy with 1-10 carbon atoms, monocyclic aromatic, alkene with 1-10. . .

32 A compound having the formula:
D]eOOBN*%%)C
wherein Xis an analog of X of Claim 29, and Kis optionally linked to the carrier protein,
B is 0, S, NH, or CH2, and
D is HP(O)OH, CH20H, P(O)(OH)2, or S03H, if D is CH20H. . .

36 A compound having the formula:
RU
R26
R
wherein X'is. an analog of X of Claim 33, and X'is optionally linked to the carrier protein,
B is 0, S, NH, or CH2,
D is P(O)OH, SO2, CHOH or SO, if D is CHOH then B is CH2, and

R24]-261 which are optionally linked to a carrier protein are
the same or different and
are H, alkyl with 2 to 22 carbon atoms, alkyl with. . .
. . .
A compound having the formula:
DI X
R2T B 000e
R28'
wherein X' is an analog of X of Claim 37, and Yis optionally
linked to the carrier
protein,
B is 0, S, NH, or CH2,
D'is P(Q), COH, provided that if D'is COH then B and Yare. . .
heteroatoms with 1-9
atoms in a linear configuration which have substituents that are phenyl,
alkyl, or alkyl with
heteroatoms, and
R2T-28'which are optionally linked to the carrier protein are
the same or different and
are H, alkyl with 2 to 22 carbon atoms, alkyl with. . .
. . .
least one of R30' and R3 V is an analog of X of Claim 41 , and said
analog
is optionally linked to a carrier protein,
D... is SO2, SO or CHOH, if D... is CHOH then Zis CH,
Z'is 0, N, or CH;. . . . or
acylamino with 1-10 carbon atoms with or without heterocyclic or phenyl
substitution, and
R29'is optionally S03H or S04H, and
RN-33' are optionally linked to a carrier protein.
. . .
48 A compound having the formula:
R37 0
R36
B0000 X
R35' CH2)n
Al
wherein Xis an analog of X of Claim 45, and is optionally linked
to carrier protein,
B is 0, S, NH, or CH2,
R34', R35% R36% and R37'are the same or different and are H,. . .
. . .
compound having the formula:
0
(CH2) x
W
JAO]El B000'
R R43t
wherein X is an analog of X of Claim 49, and X' is optionally
linked to carrier
protein,
B is 09 S, NH, or CH2,
n is an interger from 0 to 4,
R43t and R4'V are the same. . .
. . .
E31
R46- NH QI- X
wherein Q'is 0, S, NH, or CH2,
Xis an analog of X of Claim 53, and Xis optionally linked to a
carrier protein,
B'is NH or CH2, if B'is NH, then Q'is CH2, and
R45' and R46' are the same or. . . different and are H. alkyl
unsubstituted, alkyl
substituted with halogens, heteroatoms, phosphonate7 sulfonate,

carboxylate,
alkylammonium, alkene, or monocyche aromatic, and are optionally
linked to a carrier
protein.

. . .

different and are H, alkyl unsubstituted, alkyl
substituted with halogens, heteroatoms, phosphonate, sulfonate,
carboxylate,
alkylammonium, alkene, or monocyclic aromatic, and are optionally
linked to a carrier
protein.

. . .

X
wherein Q'is 0, S, NH, or CH2,
wherein X' is an analog of X of Claim 53, and X' is optionally
linked to a carrier
protein, and
R45' and R46' are the same or different and are H, alkyl unsubstituted,
alkyl
substituted with halogens, heteroatoms, phosphonate, sulfonate,
carboxylate,
alkylammonium, alkene, or monocyclic aromatic, and are optionally
linked to a carrier
protein.

. . .

different and are H, alkyl unsubstituted, alkyl
substituted with halogens, heteroatoms, phosphonate, sulfonate,
carboxylate,
alkylammonium, alkene, or monocyclic aromatic, and are optionally
linked to a carrier
protein.

63 A compound having the formula:
R47
NH
wherein X'is an analog of X of Claim 60, and which is optionally
linked to a carrier
protein,
Q'is 0, CH2, S, or NH, and
R47' and R48' are the same or different and are H, alkyl unsubstituted,
alkyl
substituted with halogens, heteroatoms, phosphonate, sulfonate,
carboxylate,
alkylammonium, alkene, or monocyclic aromatic, and are optionally
linked to a carrier
protein.

. . .

different and are H, alkyl unsubstituted,
alkyl substituted with halogens, heteroatoms, phosphonate, sulfonate,
carboxylate,
alkylammonium, alkene, or monocyclic arorn and are optionally
linked to a carrier
protein.

65 A compound having the formula-
R471
\1]
F141 NH
wherein Xis an analog of X of Claim 60, and which is optionally
linked to a carrier
protein,
Q'is CH2, and
R47' and R48' are the same or different and are H, alkyl unsubstituted,
alkyl

substituted with halogens, heteroatoms, phosphonate, sulfonate, carboxylate,
alkylammonium, alkene, or monocyclic aromatic, and are optionally linked to a carrier
protein.

. . .

different and are H, alkyl unsubstituted, alkyl
substituted with halogens, heteroatoms, phosphonate, sulfonate, carboxylate,
alkylammonium, alkene, or monocyclic aromatic, and are optionally linked to a carrier
protein.

. . .

with halogens, heteroatoms, phosphonate, sulfonate, carboxylate or alkyl ester or
alkyl amide, hydroxyl, alkylammonium, amino, alkene, or monocyclic aromatic, and are
optionally linked to a carrier protein.

. . .

having th.e formula:
ED
R511 NH
wherein Q'is 0, S, NH or CH2,
Xis an analog of X of Claim 17, and Xis optionally linked to a carrier protein,
Vis NH or CH2, if B'is NH, then Q'is CH2, and
R50' and R51' are the same or. . . with halogens, heteroatoms, phosphonate, sulfonate, carboxylate or alkyl ester or
alkyl amide, hydroxyl, alkylammonium, amino, alkene, or monocyclic aromatic, and are
optionally linked to a carrier protein.

. . .

the formula:
FtSD
R511 01] x
wherein Q'is 0, S, NH or CH2,
Yis an analog of X of Claim 67, and Yis optionally linked to a carrier protein, and
R50' and R51' are the same or different and are H, alkyl unsubstituted, alkyl
substituted with halogens, heteroatoms, phosphonate, sulfonate, carboxylate or alkyl ester or
alkyl amide, hydroxyl, alkylammonium, amino, alkene, or monocyclic aromatic, and are
optionally linked to a carrier protein.

. . .

with halogens, heteroatoms, phosphonate, sulfonate, carboxylate or alkyl ester or
alkyl amide, hydroxyl, alkylammonium, amino, alkene, or monocyclic aromatic, and are
optionally linked to a carrier protein.

. . .

the formula:
'00e
G
NH a] X
wherein Q'is 0, S, NH or CH2,
X'is an analog of X of Claim 75, and Xis optionally linked to a carrier protein,
B'is NH or CH2, if B'is NH, then Q'is CH2, and
G' is a radical of the diol. . . cycloalkyldiol or ortho-phenyldiol, and G' is optionally substituted with halogens, heteroatoms, phosphonate,
sulfonate, carboxylate, alkylammonium, alkene, or monocyclic aromatic,

and is optionally
linked to a carrier protein.

. . .

cycloalkyldiol or ortho-
phenyldiol, and G' is optionally substituted with halogens, heteroatoms, phosphonate,
sulfonate, carboxylate, alkylammonium, alkene, or monocyclic aromatic, and is optionally
linked to a carrier protein.

. . .

compound having the formula:
G
a,-)C
wherein Q'is 0, S, N-H or CH2,
Yis an analog of X of Claim 75, which is optionally linked to a carrier protein, and
G' is a radical of the diol G(OH)2, G(OH)2 is a sugar, cycloalkyldiol or ortho-
phenyldiol, and G' is optionally substituted with halogens, heteroatoms, phosphonate,
sulfonate, carboxylate, alkylammonium, alkene, or monocyclic aromatic, and are optionally
linked to a carrier protein.

. . .

cycloalkyldiol or ortho-
phenyldiol, and G' is optionally substituted with halogens, heteroatoms, phosphonate,
sulfonate, carboxylate, alkylammonium, alkene, or monocyclic aromatic, and are optionally
linked to a carrier protein.

. . .

with halogens, heteroatoms, phosphonate, sulfonate, carboxylate or alkyl ester or alkyl amide, hydroxyl, alkylammonium, amino, alkene, or monocyclic aromatic, and
are optionally linked to a carrier protein.

. . .

87 A compound having the formula:
52,
\]-
R53 NH
wherein X' is an analog of X of Claim 82, and which is optionally linked to a carrier
protein,
Q'is CH2 or NH, and
R52' and R53' are the same or different, and are H, alkyl unsubstituted, alkyl
substituted. . . with halogens, heteroatoms, phosphonate, sulfonate, carboxylate or alkyl ester or
alkyl an-dde, hydroxyl, alkylammonium, amino, alkene, or monocyclic aromatic, and are
optionally linked to a carrier protein.

. . .

with halogens, heteroatoms, phosphonate, sulfonate, carboxylate or alkyl ester or alkyl amide, hydroxyl, alkylammonium, amino, alkene, or monocyclic aromatic, and
are optionally linked to a carrier protein.

89 A compound having the formula:
521
at X
R530
wherein X'is an analog of X of Claim 82, and which is optionally linked to a carrier
protein,

Q'is CH2, and
R52t and R53' are the same or different, and are H, alkyl unsubstituted, alkyl
substituted with halogens, heteroatoms, phosphonate, sulfonate, carboxylate or alkyl ester or
alkyl amide, hydroxyl, alkylammonium, amino, alkene, or monocyclic aromatic, and are
optionaE v linked to a carrier protein.

93 A compound having the formula:
00000
G'
NH
wherein Yis an analog of X of Claim 90, and which is optionally linked to a carrier
protein,
Q'is CH2 or NH, and
G' is a radical of the diol G(OH)2, G(OH)2 is a sugar, cycloalkyldiol or ortho-
phenyldiol, and G' is optionally substituted with halogens, heteroatoms, phosphonate,
sulfonate, carboxylate, alkylammonium, alkene, or monocyclic aromatic, and is optionally
linked to a carrier protein.

. . .

cycloalkyldiol or ortho-
phenyldiol, and G' is optionally substituted with halogens, heteroatoms, phosphonate,
sulfonate, carboxylate, alkylammonium, alkene, or monocyclic aromatic, and is optionally
linked to a carrier protein, and
R59' is H, alkyl unsubstituted, alkyl substituted with halogens, heteroatoms,
phosphonate, sulfonate, carboxylate or alkyl ester or alkyl amide, hydroxyl, alkylammonium,
amino, alkene, or monocyclic aromatic, and is optionally linked to a carrier protein.

95 A compound having the formula:
NH
wherein Xis an analog of X of Claim 90, and which is optionally linked to a carrier
protein,
Q'is CH2, and
G' is a radical of the diol G(OH)2, G(OH)2 is a sugar, cycloalkyldiol or ortho-
phenyldiol, and G' is optionally substituted with halogens, heteroatoms, phosphonate,
sulfonate, carboxylate, alkylammonium, alkene, or monocyclic aromatic, and is optionally
linked to a carrier protein.

. . .

cycloalkyldiol or ortho-
phenyldiol, and G' is optionally substituted with halogens, heteroatoms, phosphonate,
sulfonate, carboxylate, alkylammonium, alkene, or monocyclic aromatic, and is optionally
linked to a carrier protein.

. . .

X is a radical of the drug XQH, where Q is 0 or NH, and
V is a hexopyranose or hexofaranose conjugated to QX via the anomeric position of
the sugar with optional alpha or beta configuration.

. . .

nucleoside analog or phosphoran-dde
mustard [HOP(O)(NH2)N(CH2CH2CI)21, melphalan or doxorubicin.
I 00. A compound as in Claim - wherein V is Glucose, Glucosamine, D-Quinovopyranose,
Galactose, Galactosamine, L-Fucopyranose, L- Rhamnopyranose, D-Glucopyranuronic acid,
D-Galactopyranuronic acid, D-manopyranuronic acid, or D-lodopyranuronic ac id.
101. Acompoundhavingthefonnula:
N
z 01]x
M
wherein Xis an analog of X of Claim 97, and which is optionally linked to a carrier
protein,
Q'is NH, and
M' is a 1,4-diradicaI of a n-pentane where Cl, C2, C3 and C5 are optionally
substituted with OH, and Mis optionally linked to a carrier protein.
102. A compound having the formula:
NH
/-]S
M' is a 1,4-diradical of a n-pentane where Cl, C2, C3 and C5 are optionally
substituted with OH, and M'is optionally linked to a carrier protein.
103. A compound having the formula:
NH
I (t])c
wherein X'is an analog of X of Claim 97, and which is optionally linked to a carrier
protein,
Q'is CH2, and
M' is a 1,4-diradical of a n-pentane where Cl, C2, C3 and C5 are optionally
substituted with OH, and M' is optionally linked to a carrier protein.
104. A prodrug comprising a compound as claimed in Claim 1.
105. A pharmaceutical composition comprising:
.(a) an effective. . . 2;
VL antibody I-S-VH antibody 1-S-VL antibody 2-S-VH antibody 2;
VL antibody I-S-VH antibody I-S-VH antibody 2-S-VL antibody 2;
wherein -S- is a linker sequence; and
(ii) isolating said bispecific antibody.
125. A method as in Claim 123 wherein antibody 1 is an antibody capable of. . . I -S-VL antibody 2,
(iii) combining the products of steps (i) and (h), and
(iv) isolating said bispecific antibody,
wherein -S- is a linker sequence.
127. Amethodofsynthesizingabispecificantibodycomprisingthestepsof-
(i) expressing a gene having the sequence;
VL antibody 2-S-VH antibody 1, and
(ji) expressing a gene having the sequence:
VH antibody 2-S-VL antibody 1,
(iii) combining the products of steps (i) and (ii), and
(iv) isolating said bispecific antibody,
wherein -S- is a linker sequence.
128. A compound having the formula:
R 60o 0
N 11
R]Id CHCH2CH20P(N(CH2CH2CI)2)2
wherein R60 and R61 are the same or different and independently. . .
substituted aromatic compound having the formula:

R67
R68 R66
N+oe A-
R B-X
wherein X is a radical of the drug XOH and is optionally linked to a carrier protein;
B is 0, S, NH or CH2;
D is HOP(O), S02, CHOH or SO (with any stereochemistry);
R671 R689. . . or alkylammonium, and wherein
A- is an anion.
131. A therapeutic combination as recited in claim 111 wherein said immunoconjugate
is modified by conjugation of a plurality of nonantigenic molecules to the
immunoconjugate.
132. A therapeutic combination as recited in claim 112 wherein said immunoconjugate
is modified by conjugation of a plurality of nonantigenic, molecules to the
immunoconjugate.
SU.BSTITUTE SHEET

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FULL ESTIMATED COST	16.43	20.32

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FILE LAST UPDATED: 20 Feb 2007 (20070220/ED)

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http://www.cas.org/infopolicy.html

=> s antibod?
L26 484149 ANTIBOD?

=> s enzyme?
L27 1013392 ENZYME?

=> s l26 (4W)l27
L28 7572 L26 (4W)L27

=> s ?glucuronidase
L29 16687 ?GLUCURONIDASE

```
=> s l28 and l29
L30         93 L28 AND L29

=> s CEA or (Carcinogenic Embryonic Antigen)
          6400 CEA
           195 CEAS
          6573 CEA
                 (CEA OR CEAS)
         29571 CARCINOGENIC
            26 CARCINOGENICS
         29582 CARCINOGENIC
                 (CARCINOGENIC OR CARCINOGENICS)
         79153 EMBRYONIC
             3 EMBRYONICS
         79154 EMBRYONIC
                 (EMBRYONIC OR EMBRYONICS)
        306825 ANTIGEN
        244667 ANTIGENS
        387260 ANTIGEN
                 (ANTIGEN OR ANTIGENS)
             3 CARCINOGENIC EMBRYONIC ANTIGEN
                 (CARCINOGENIC(W)EMBRYONIC(W)ANTIGEN)
L31        6574 CEA OR (CARCINOGENIC EMBRYONIC ANTIGEN)

=> s l31 and l30
L32         7 L31 AND L30

=> d ibib 1-7

L32  ANSWER 1 OF 7  CAPLUS  COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER:         1998:546530  CAPLUS
DOCUMENT NUMBER:          129:254559
TITLE:                    Prodrugs of anthracyclines for use in antibody
                          -directed enzyme prodrug therapy
AUTHOR(S):                Florent, Jean-Claude; Dong, Xia; Gaudel, Gilbert;
                          Mitaku, Sofia; Monneret, Claude; Gesson, Jean-Pierre;
                          Jacquesy, Jean-Claude; Mondon, Martine; Renoux,
                          Brigitte; Andrianomenjanahary, Solo; Michel, Sylvie;
                          Koch, Michel; Tillequin, Francois; Gerken, Manfred;
                          Czech, Joerg; Straub, Rainer; Bosslet, Klaus
CORPORATE SOURCE:         Section Recherche, UMR 176 CNRS/Institut Curie, Paris,
                          F-75248, Fr.
SOURCE:                   Journal of Medicinal Chemistry (1998), 41(19),
                          3572-3581
                          CODEN: JMCMAR; ISSN: 0022-2623
PUBLISHER:                American Chemical Society
DOCUMENT TYPE:            Journal
LANGUAGE:                 English
REFERENCE COUNT:          41    THERE ARE 41 CITED REFERENCES AVAILABLE FOR THIS
                                RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L32  ANSWER 2 OF 7  CAPLUS  COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER:         1997:461628  CAPLUS
DOCUMENT NUMBER:          127:104332
TITLE:                    Cell-targeted cytotoxic drug therapy system, and
                          preparation of associated compounds
INVENTOR(S):              Khan, Tariq
PATENT ASSIGNEE(S):       Aepact Limited, UK; Khan, Tariq
SOURCE:                   PCT Int. Appl., 84 pp.
                          CODEN: PIXXD2
DOCUMENT TYPE:            Patent
LANGUAGE:                 English
FAMILY ACC. NUM. COUNT:   1
PATENT INFORMATION:
```

PATENT NO.	KIND	DATE	APPLICATION NO.		DATE
WO 9720580	A1	19970612	WO 1996-GB3000		19961206
W: CA, GB, JP, US					
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE					
CA 2239203	A1	19970612	CA 1996-2239203		19961206
EP 865298	A1	19980923	EP 1996-940685		19961206
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI					
JP 2000502071	T	20000222	JP 1997-521082		19961206
PRIORITY APPLN. INFO.:			GB 1995-24942	A	19951206
			WO 1996-GB3000	W	19961206

OTHER SOURCE(S): MARPAT 127:104332

L32 ANSWER 3 OF 7 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1995:412729 CAPLUS
DOCUMENT NUMBER: 122:151369
TITLE: Modified glycosidation of fusion proteins of anti-tumor antibodies and prodrug activating enzymes and the use of the proteins in the targetted treatment of tumors
INVENTOR(S): Bosslet, Klaus; Czech, Joerg; Hoffmann, Dieter
PATENT ASSIGNEE(S): Behringwerke AG, Germany
SOURCE: Eur. Pat. Appl., 28 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.		DATE
EP 623352	A2	19941109	EP 1994-106394		19940425
EP 623352	A3	19950222			
EP 623352	B1	20020724			
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, NL, PT, SE					
DE 4314556	A1	19941110	DE 1993-4314556		19930504
AT 220921	T	20020815	AT 1994-106394		19940425
ES 2177554	T3	20021216	ES 1994-106394		19940425
PT 623352	T	20021231	PT 1994-106394		19940425
AU 9461829	A	19941110	AU 1994-61829		19940502
AU 684750	B2	19980108			
CA 2122745	A1	19941105	CA 1994-2122745		19940503
JP 06319554	A	19941122	JP 1994-117524		19940506
US 2004202646	A1	20041014	US 2004-815925		20040402
PRIORITY APPLN. INFO.:			DE 1993-4314556	A	19930504
			EP 1994-106394	A	19940425
			US 1994-235395	B1	19940429
			US 1996-663406	B1	19960613
			US 1999-302434	B1	19990430

L32 ANSWER 4 OF 7 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1993:508496 CAPLUS
DOCUMENT NUMBER: 119:108496
TITLE: Molecular and functional characterization of a fusion protein suited for tumor specific prodrug activation
AUTHOR(S): Bosslet, K.; Czech, J.; Lorenz, P.; Sedlacek, H. H.; Schuermann, M.; Seemann, G.
CORPORATE SOURCE: Behring Res. Lab., Marburg, W-3550, Germany
SOURCE: Monoclonal Antibodies 2 (1993), 205-17. Editor(s): Epenetos, Agamemnon A. Chapman and Hall: London, Uk.
CODEN: 59CLAZ
DOCUMENT TYPE: Conference

LANGUAGE: English

L32 ANSWER 5 OF 7 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1993:66829 CAPLUS
DOCUMENT NUMBER: 118:66829
TITLE: Fusion proteins of monoclonal antibodies and β-glucuronidase for site-specific prodrug activation
INVENTOR(S): Seemann, Gerhard; Bosslet, Klaus; Czech, Joerg; Kolar, Cenek; Hoffmann, Dieter; Sedlacek, Hans Harald
PATENT ASSIGNEE(S): Behringwerke A.-G., Germany
SOURCE: Eur. Pat. Appl., 34 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 4
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 501215	A2	19920902	EP 1992-102197	19920210
EP 501215	A3	19930811		
EP 501215	B1	20000524		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, PT, SE				
DE 4106389	A1	19920903	DE 1991-4106389	19910228
AT 193326	T	20000615	AT 1992-102197	19920210
PT 501215	T	20001031	PT 1992-102197	19920210
ES 2149159	T3	20001101	ES 1992-102197	19920210
AU 9211251	A	19930128	AU 1992-11251	19920227
AU 660445	B2	19950629		
CA 2062047	A1	19920829	CA 1992-2062047	19920228
JP 07179500	A	19950718	JP 1992-78644	19920228
JP 3524933	B2	20040510		
GR 3033708	T3	20001031	GR 2000-401398	20000616

PRIORITY APPLN. INFO.: DE 1991-4106389 A 19910228

L32 ANSWER 6 OF 7 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1992:241811 CAPLUS
DOCUMENT NUMBER: 116:241811
TITLE: Molecular and functional characterization of a fusion protein suited for tumor specific prodrug activation
AUTHOR(S): Bosslet, K.; Czech, J.; Lorenz, P.; Sedlacek, H. H.; Schuermann, M.; Seemann, G.
CORPORATE SOURCE: Res. Lab., Behringwerke A.-G., Marburg, W-3550, Germany
SOURCE: British Journal of Cancer (1992), 65(2), 234-8
CODEN: BJCAAI; ISSN: 0007-0920
DOCUMENT TYPE: Journal
LANGUAGE: English

L32 ANSWER 7 OF 7 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1991:605931 CAPLUS
DOCUMENT NUMBER: 115:205931
TITLE: Method for antibody targeting of diagnostic or therapeutic agents
INVENTOR(S): Hansen, Hans J.
PATENT ASSIGNEE(S): Immunomedics, Inc., USA
SOURCE: Can. Pat. Appl., 49 pp.
CODEN: CPXXEB
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
CA 2005162	A1	19910611	CA 1989-2005162	19891211
PRIORITY APPLN. INFO.:			CA 1989-2005162	19891211